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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/990,185	11/21/2001	Krzysztof Palczewski	P-NS 4970	1224

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EXAMINER	
ANGELL, JON E	
ART UNIT	**PAPER NUMBER**
1635	

DATE MAILED: 06/14/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/990,185	PALCZEWSKI ET AL.	
	Examiner	Art Unit	
	Jon Eric Angell	1635	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *23 March 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-27,30-36 and 39* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-27,30-36 and 39* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *21 November 2001* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

5.0-0

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 3/23/2005 has been entered. Claims 1-27, 30-36 and new claim 39 are currently pending in the application and are addressed herein.

Applicant's arguments are addressed on a per section basis. The text of those sections of Title 35, U.S. Code not included in this Action can be found in a prior Office Action. Any rejections not reiterated in this action have been withdrawn as being obviated by the amendment of the claims and/or applicant's arguments.

Claim Rejections - 35 USC § 112

Claims 1-27, 30-36 and 39 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 USC 112, first paragraph, have been described by the court in *In re Wands*, 8 USPQ2d 1400 (CA FC 1988).

Wands states on page 1404,

"Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in Ex parte Forman. They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims."

The instant claims are drawn to a gene targeting construct, a vector comprising the construct, a cell comprising the construct, a mouse cell whose genome comprises a functional disruption of one or both rhodopsin gene alleles and a mouse whose genome comprises a functional disruption of one or both rhodopsin gene alleles and a transgene encoding a polypeptide comprising a ROS targeting signal operably associated with a rod-specific regulatory sequence wherein the polypeptide is not a rhodopsin (and claim 39 indicates that sufficient expression results from said transgene to produce an encoded polypeptide).

It is noted that the only use for the construct, vector and mouse cell contemplated by the specification is for making a transgenic mouse comprising a functional disruption of one or both endogenous rhodopsin gene alleles wherein the mouse expresses a polypeptide of interest comprising an rod outer segment (ROS) targeting signal. It is respectfully pointed out that the claims drawn to a transgenic mouse do not indicate that the transgene is inserted into the rhodopsin allele(s). Therefore, given the broadest reasonable interpretation, the claim encompass a mouse having: (1) a functional disruption of one or both rhodopsin gene alleles, and (2) a transgene that is inserted into the genome either by homologous recombination into the rhodopsin gene or by random integration into the genome.

The specification contemplates two potential uses for the transgenic mouse: 1) as a bioreactor to express large quantities of the transgene protein in the rod outer segment of the eye,

and 2) as a research tool wherein the mouse expresses a transgene encoding a GPCR in the rod outer segment of the eye such that the mouse can be used to identify modulators of a GPCR activity. In both contemplated uses, the mouse must express the transgene protein such that it is properly localized to the rod outer segment of the eye. The transgenic mouse has utility as a research tool to identify modulators of a GPCR activity. However, the specification does not provide an enabling disclosure for the claimed invention (either as a bioreactor or as a research tool) in view of the state of the art at the time of filing which indicates that it would be unpredictable to make the claimed transgenic mouse such that the mouse expressed the transgenic protein in the rod outer segment (ROS) of the eye at a sufficient level to be used as a bioreactor or that a functional transgenic protein would be properly expressed in the ROS at a sufficient level such that the mouse could be used as research tool. In order for the mouse (and thus the nucleic acid construct, vector and cell) to be enabled the transgenic mouse MUST express the transgene of interest such that the transgenic protein is expressed in the ROS of the eye in a functional form and at a concentration sufficient for performing the drug screening assays or at a concentration sufficient to enable the transgenic mouse as a bioreactor.

However, with respect to the mouse as a bioreactor, there is no disclosure indicating that a transgenic mouse has been made; therefore, there is no demonstration that the transgene protein would be properly expressed and localized to the rod outer segment of the eye at a sufficient concentration such that the transgenic protein could be purified from the eye of mouse. With respect to the transgenic mouse as a tool to identify modulators of GPCRs, there is no disclosure indicating that the transgenic mouse has been made; therefore, there is no demonstration that a functional GPCR could be properly expressed and localized to the rod outer segment of the eye.

The specification fails to provide an enabling disclosure for the preparation of the claimed transgenic mouse that expresses a functional transgenic protein specifically in the rod outer segment of the eye at a sufficient concentration for performing drug screening assays or for protein purification. Therefore, undue experimentation would have been required for one of skill in the art to make and/or use the claimed invention.

Note that the mere capability to perform gene transfer in a mouse is not enabling because the desired expression of the transgene cannot be predictably achieved by simply introducing transgene constructs of the types recited in the claims. While gene transfer techniques are well developed for a number of species, and particularly for the mouse, methods for achieving the desired level of transgene expression in appropriate tissues are less well established. The introduction of DNA into the mammalian genome can ordinarily be achieved most reliably by microinjection or retrovirus-mediated gene transfer. However, the state of the art for transgenic animals is unpredictable because the method of gene transfer typically relies on random integration of the transgene construct. Insertional inactivation of endogenous genes and position effects can dramatically influence the phenotype of the resultant transgenic animal [see Ryan *et al.*, **Sem. Neph.** 22:154-160, 2002; previously cited]. Ryan states that methods such as pronuclear injection or gene targeting by homologous recombination are still limited by several unpredictabilities, including differences in transgene copy number and position of integration into the genome. Furthermore, Ryan teaches,

> "The location of integration can have dramatic effects on the expression of a transgene. Called the position effect, transcriptional regulatory sequences at or near the insertion site can strongly influence your transgene, even impart a new set of instructions." [See p. 155, 2nd column]; and,

> "Disadvantages include differences in transgene copy number and position of integration in the genome. Indeed, there is essentially no control over the number of transgenes that integrate into the genome. Although there is generally no direct correlation between transgene copy number and the level of expression, high-level supraphysiologic expression of transgene is possible, calling into question the physiologic relevance of such a model." (See p. 155, second column); and,
>
> "It is very important to recognize that each of the models described herein have some potential limitations and that no model will perfectly emulate a gene at its normal location in the genome. Moreover, in the end a phenotype observed in these experiments is not only the consequence of the manipulation mode, but the genetic background of the animals being studied. Numerous differences in baseline phenotypes, such as blood pressure and tumor susceptibility, have been reported in different inbred strains of mice. Although most ES cells used in gene targeting are derived from the 129 inbred mouse strain, they are re-implanted into (2578116) blastocysts and then bred with C578176, thus, forming a mixed genetic background. Until 7 to 10 rounds (3-4 years) of successive backcross breading is performed, it is crucial to use non-transgenic littermates as the control animals for any experiment to ensure that the experimental differences do not result from influences of genetic background. This is particularly important when one considers our recent data showing that the 129 strain has a genetic defect in relaxation of the aorta in response to an endothelial-dependent agonists." (See p. 159, first column).

In the instant case the claims encompass a mouse having a functional disruption of the rhodopsin gene (either one or both alleles of rhodopsin) as well as another functional transgene that can be integrated randomly into the genome or directly into the rhodopsin gene by homologous recombination. The integrated transgene then theoretically be under the control of gene expression elements (i.e. promoters, enhancers, etc.) present at the site of integration. Furthermore, the transgene of interest comprises a ROS targeting sequence in order to theoretically target the expressed transgenic polypeptide to the rod outer segments where the transgenic protein can be purified or used in drug screening assays.

However, expressing the transgenic protein specifically in the rod outer segment of the eye in a mouse comprising a functional disruption of one or both rhodopsin gene alleles may not be possible. Lem et al. **[PNAS Vol. 96p. 736-741; 1999]** teaches,

> "Retinas in mice lacking both opsin alleles initially developed normally, except that rod outer segments failed to form. Within months of birth, photoreceptor cells degenerated completely." (See abstract).

Therefore, functional disruption of the rhodopsin gene may result in a mouse that fails to develop rod outer segments. This would create a problem for expressing the transgenic protein having an ROS targeting sequence. It is unclear how the transgenic protein would be expressed and targeted to the ROS in a mouse where the ROS fails to form. With respect to mice comprising a knockout of a single rhodopsin gene, it appears that these mice do develop a rod outer segment. However, Lem teaches that the rod outer segment development is retarded, that older animals exhibited retinal degeneration (e.g., see page 741, third full paragraph), and that "mis-oriented outer segments" were observed (e.g., see page 739: Figure 4 and right side column third full paragraph). Therefore, it is unclear if the transgenic protein could be targeted to the rod outer segment in a mouse comprising a disruption of a single rhodopsin. It is noted that the claims encompass expressing a GPCR in the ROS and the specification indicates such mouse can be used to screen drugs that modulate the activity of the GPCR. However, it is cannot be predicted if the transgenic GPCR protein would be correctly oriented (i.e., with the extracellular domain on the outer side of the cell) in a mis-oriented ROS. Should the GPCR protein be mis-oriented in the ROS, it would not be a functional GPCR and the mouse would not be used for identifying modulators of the GPCR as disclosed in the specification.

Furthermore, the development of the eye is a complicated process involving the precise interaction of many different gene products and it is unclear how the degenerative effect of functionally inhibiting one or both rhodopsin gene alleles in a mouse would effect the expression and localization of a transgene in the eye without performing additional experimentation.

Therefore, without actually making the transgenic mouse, it cannot be predicted that the transgene will be expressed at the desired level or at the specific location (i.e., the ROS of the eye) such that the mouse could predictable be used as a bioreactor or a drug-screening tool.

Additionally, expression of the transgene and the effect of transgene expression on the transgenic animal depend upon the particular gene construct used, to an unpredictable extent. This is supported by Holschneider *et al.* [**Int J. Devl. Neuroscience** 18:615-618, 2000] who indicate that,

> "[The] knocking out or insertion of a single gene may result in no phenotypic change. This may be the case, in particular, if there exist gene redundancy mechanisms whose presence may prevent abnormal phenotypes from becoming masked. Conversely, single genes are often essential in a number of different behaviors and physiologic processes. Hence, ablation of an individual gene may prove so drastic as to be lethal, or so widespread as to create an amalgam of phenotypes whose interpretation becomes confounded by the interactions of the various new physiologic changes or behaviors." [See p. 615, col. 1-2].

Holschneider discusses various factors that contribute to the resulting phenotype of transgenic mice, including compensatory systems which may be activated to mask the resulting phenotype, these compensatory changes may be due to the differential expression of another gene, which may be regulated by the downstream product of the ablated gene, as well as the variability in phenotypic characterization due to particular mouse strains [see p. 616, 1st column]. Therefore, it is unpredictable a mouse comprising a disruption of one or both rhodopsin genes and further comprising an insertion of a transgene designed to express the transgenic protein in the ROS would be able to express the transgenic protein in the ROS of the eye such that the mouse could be used as a bioreactor or as a drug screening tool.

In view of the teachings of Lem, Ryan and Holschneider as a whole it is clear that it would not be a matter of routine experimentation to make a transgenic mouse that has a

functional disruption of one or both rhodopsin genes wherein the mouse further expresses a transgenic protein of interest such that the protein specifically localizes to the ROS. Furthermore, the amount of experimentation required to be able to make the claimed mouse is considered to be undue in view of the problems recognized in the art, especially the teaching that disruption of the rhodopsin gene results in the failure of the outer segment to form (in mice comprising disruption of both rhodopsin alleles) or in mis-oriented ROS (in mice comprising a disruption of a single rhodopsin allele).

Considering that the only contemplated use for the targeting construct, the vector, and the cell comprising the construct is for making the transgenic mouse, these claims are also rejected as not being enabled because the only use contemplated is not enabled.

Response to Arguments

Applicant's arguments filed 3/23/2005 have been fully considered but they are not persuasive.

Applicants reiterate that the law is clear with respect to undue experimentation: Routine experimentation, even if it is time consuming, does not constitute undue experimentation. *Johns Hopkins Univ. v. Cellpro, Inc,* 152 F.3d 1342, 1360 (Fed. Cir. 1998).

With respect to the teachings of Ryan, Applicants maintain that Ryan does not state or suggest that expression is not unpredictable. Applicants contend that Ryan indicates that transgenic expression by random genome insertion is associated with certain disadvantages such as "differences in transgene copy number and position of integration in the genome" and "differences in baseline phenotypes" reported in different genetic backgrounds. Aplicants also

assert that the reported disadvantages in Ryan et al. are stated in context of constructing an animal model that perfectly emulates a gene at its normal location in the genome and that such descriptions do not amount to undue experimentation for making and using the invention as claimed and that production of an animal model that perfectly emulates a gene at it's normal locus differs from the purpose of the claimed invention. (See pages 6-7 of the respnse filed 3/23/2005).

Applicants also contend that the constructs, cells and mice of the claimed invention place the transgene in a normal locus for rhodopsin such that the claimed flanking sequences avoid the deficiencies described by Ryan et al. and render its description inapplicable as support for unpredictability and any description in Ryan et al. regarding unpredictability is associated with random transgenic expression, which is distinct in both the method of insertion and in the obtained results. Accordingly, the claimed cell and mouse are not fraught with disadvantages such as valiable transgene copy number and position of integration observed using random insertion methods which led to variation in exjression levels described by Ryan et al.

With respect to the teachings of Lem, Applicants argue that Lem indicates that retinas in mice lacking both opsin alleles initially developed normally and any later stage retinal development problems can be circumvented by constructing a single rhodopsin gene knock-out instead of both alleles.

With respect to the teachings of Holschneider, Applicants argue that the reference is nonanalogous to the claimed invention because the application describes and claims the construction of a transgene encoding polypeptide having a ROS target signal flanked by

sequences for homologous recombination that is operable association with a rod-specific regulatory signal and that the reference is concerned with observing changes in phenotype.

In response it is acknowledged that routine experimentation, even if it is time consuming, does not constitute undue experimentation.

With respect to Applicants arguments pertaining to the teachings of Ryan, Lem and Holschneider, it is respectfully pointed out that the rejection is based on the the teachings of the three references as a whole, and not just on the individual teachings of any one reference.

With respect to the teaching of Lem, it is respectfully pointed out that the complete statement referred to by Applicants is "Retinas in mice lacking both opsin alleles initially developed normally, **except that rod outer segments failed to form**." (see abstract; also see page 741, first full paragraph). Therefore, it is clearly unpredictable that the claimed mice having functional disruption of both rhodopsin alleles would be able to specifically express the transgenic protein in the rod outer segments of this mouse as the rod outer segments failed to form. Furthermore, as indicated above, it is acknowledged that mice having just one rhodopsin gene disrupted appear to have developed a rod outer segment. However, Lem teaches indicate that the rod outer segment development of the single allele knock-out was retarded, that older animals exhibited retinal degeneration (e.g., see page 741, third full paragraph), and that "mis-oriented outer segments" were observed in the single knock out mice (e.g., see page 739: Figure 4 and right side column third full paragraph).

With respect to the teaching of Ryan, it is respectfully pointed out that the instant claims are not limited to a mouse having the transgene specifically integrated into the rhodopsin gene.

As such, the claims encompass a mouse having a functional disruption of the rhodopsin gene and further comprising a transgene of interest wherein the transgene can be integrated anywhere in the genome (such as by random integration). As indicated above, Ryan indicates that the location of integration can have dramatic effects on the expression of a transgene because transcriptional regulatory sequences at or near the insertion site can strongly influence the transgene. The teaching of Ryan is relevant to the instant case because it indicates that integration the position effect is critical for transgene expression. The position effect is important to consider in the instant case because should the transgene integrate into a site that does not confer expression in the eye, then the transgene would not be expressed in the ROS. Therefore, Ryan does imply that specific, targeted expression of a randomly integrated transgene is unpredictable.

With respect to the teaching of Holschneider, it is respectfully pointed out that the instant claims are not limited to a mouse having the transgene specifically integrated into the rhodopsin gene. As such, the claims encompass a mouse having a functional disruption of the rhodopsin gene and further comprising a transgene of interest wherein the transgene can be integrated anywhere in the genome (such as by random integration) such that the transgene would not be in operable association with a rod-specific regulatory signal. Furthermore, with respect to Applicants assertion that Holschneider is concerned with changes in phenotype, it is respectfully pointed out that the reference is relevant to the instant case because it indicates that disruption of one or both alleles of a gene can result in unexpected phenotypic changes in the mouse. The phenotypic changes relevant to the instant case are the phenotypic changes related to the disruption of one or both rhodopsin alleles and the effect that the phenotypic changes may have

on proper expression of the transgenic protein in the ROS, especially in view of the teachings of Lem, as indicated above.

Therefore, Applicants arguments are not persuasive and the rejection is proper.

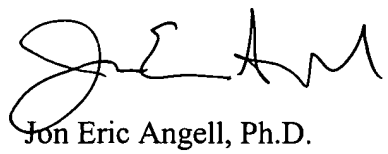
Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jon Eric Angell whose telephone number is 571-272-0756. The examiner can normally be reached on Mon-Fri, with every other Friday off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, John LeGuyader can be reached on 571-272-0760. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).



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Art unit 1635